Cancer Cell
Previews

IDH1 Mutations in Gliomas: When an Enzyme Loses Its Grip

Christian Frezza,[1] Daniel A. Tennant,[1] and Eyal Gottlieb[1,*]
[1]Cancer Research UK, The Beatson Institute for Cancer Research, Glasgow G61 1BD, UK
*Correspondence: e.gottlieb@beatson.gla.ac.uk
DOI 10.1016/j.ccr.2009.12.031

The growing interest in cancer metabolism is best demonstrated by the rapid progress made in studying isocitrate dehydrogenase (IDH) mutations since their discovery just over a year ago. In a recent study published in *Nature*, Dang et al. identified 2-hydroxyglutarate as a product of tumor-associated IDH mutants with potential oncogenic activities.

It is now almost a century since the studies that first associated cellular metabolic changes with cancer. However, the recognition of a causal connection between metabolic alterations and cancer formation was revealed only this decade. Ironically, it was genetics, rather than biochemistry, that enabled this breakthrough when genes encoding mitochondrial enzymes of the tricarboxylic acid (TCA) cycle, succinate dehydrogenase (SDH) and fumarate hydratase (FH), were identified as bona fide tumor suppressors (King et al., 2006). Over the past year, new genetic studies placed another metabolic enzyme, isocitrate dehydrogenase (IDH), in the spotlight of cancer biology (Yan et al., 2009a). High-throughput sequencing revealed that two of the three isoforms of IDH (IDH1 and IDH2) are mutated in high proportions in gliomas (Parsons et al., 2008; Yan et al., 2009b). However, unlike SDH and FH, IDH mutations do not follow Knudson's two-hit model of tumor suppressor genes. In the new study, Dang et al. (2009) demonstrated that although IDH1 mutants lose their normal enzymatic activity in tumors, they gain a new one, generating a new product, 2-hydroxyglutarate, with potentially tumor-supporting actions (making it an onco-metabolite).

Eukaryotic cells contain two classes of IDH enzymes according to dependence on either NAD^+ or $NADP^+$. These enzymes normally convert isocitrate to α-ketoglutarate (aka 2-oxoglutarate), with the concurrent reduction of $NAD(P)^+$ to NAD(P)H (Figure 1). The two $NADP^+$-dependent forms, IDH1 and IDH2, are cytosolic and mitochondrial, respectively. IDH3, the only NAD^+-dependent IDH, is located at the mitochondria and is part of the TCA cycle. Rapid cycling of metabolites between cytosol and mitochondria is a common feature of cellular metabolism. Metabolites entering the mitochondria can be processed for energy generation usually through the production of NADH in the TCA cycle whereas metabolites exported back to the cytosol take part in anabolic processes. The transport of metabolites is also coupled to electron exchange between mitochondrial and cytosolic NADH and NADPH, both of which cannot move across the mitochondrial inner membrane (Figure 1). Because mitochondrial NADH operates in energy metabolism and cytosolic NADPH functions in anabolic processes and redox control, it is reasonable to expect changes in one or all of these processes in tumors carrying an IDH mutation.

Until now, only mutations in IDH1 and 2 were found in cancers, therefore leaving the TCA cycle untouched (Yan et al., 2009a). IDH1 mutations form the lion's share of IDH mutations found in cancer, with IDH2 mutation being much less common. So far, gliomas have been shown as the cancer type most likely to contain IDH mutations. Interestingly, they seem to arise early in the development of a glioma, suggesting that it confers advantage early on in tumor progression. One of the most striking features of IDH1 and 2 mutations is that it is always the same residue that is mutated: R132 in IDH1 and R172 in IDH2. These residues create the hydrophilic interactions that allow the binding of isocitrate (Xu et al., 2004). The residues that are substituted for arginine are wide ranging, which strongly suggests that it is not the new residue, but the replacement of the arginine, which supports tumorigenesis by impairing isocitrate binding. Indeed, loss of IDH function was reported for these mutants and therefore IDH was suggested to be a tumor suppressor (Zhao et al., 2009). However, the fact that mutations were observed only on specific arginine residues and only on one allele of *IDH1/2* with the other remaining wild-type (WT) led to the hypothesis that these are, in fact, gain- rather than loss-of-function mutations with oncogenic potential.

The new work (Dang et al., 2009) started with large-scale metabolite quantification (metabolomics) of cells expressing either WT or tumor-derived mutant of IDH1 (R132H). Only one significant metabolic change was observed in mutant-IDH1-expressing cells, which was a large accumulation of 2-hydroxyglutarate, a reduced form of α-ketoglutarate (Figure 1). Indeed, Dang et al. confirmed that the carbon backbone of the accumulated 2-hydroxyglutarate is derived from glutamine, the major source of α-ketoglutarate in these cells (Figure 1). These results suggest that the mutant IDH1 changed its substrate specificity and directionality. In vitro enzymatic analysis confirmed this; whereas WT IDH1 converted isocitrate to α-ketoglutarate, several tumor-associated mutants of IDH1 could no longer catalyze this reaction and instead reduced α-ketoglutarate to 2-hydroxyglutarate (but not to isocitrate). Structural comparison of the mutant and WT IDH1 revealed that mutations in R132 change the orientation of the catalytic site so the enzyme binds NADPH with higher affinity, a feature that supports reductase rather than

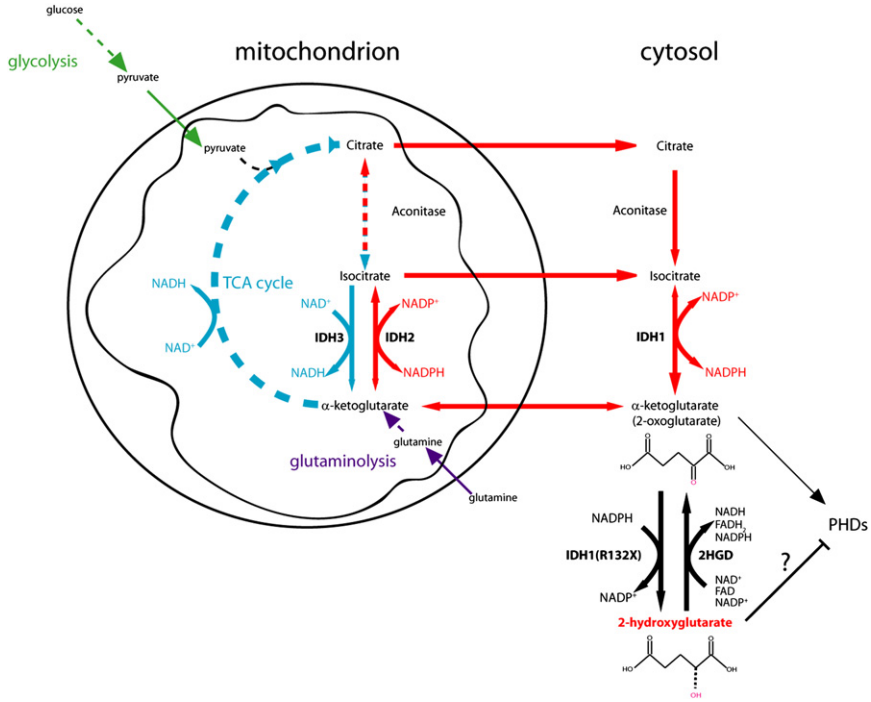

Figure 1. The Roles of IDH Enzymes in the Exchange of Metabolites between the Mitochondria and the Cytosol, and Their Potential Role in Tumorigenesis

Many isoforms of TCA cycle enzymes (light blue) also operate in the cytosol. They are important for synchronizing bioenergetic and anabolic needs by directing TCA cycle metabolites and electrons, in the forms of NAD(P)H, in and out the mitochondria (red). The two major carbon sources for these metabolites are glucose and glutamine, which are catabolized via glycolysis (green) and glutaminolysis (purple), respectively. The three IDH isoenzymes are important players in these processes. IDH3 is part of the TCA cycle where it generates NADH as a fuel for energy production while IDH1 and 2 are important for shuttling electrons between the mitochondria and the cytosol. Although mutations in IDH1 are expected to hinder these processes, newly described work (Dang et al., 2009) proposes a new gain-of-function role for glioma-associated mutants of IDH1. R132 mutations of IDH1 generate a new enzyme with α-ketoglutarate reductase activity that produces 2-hydroxyglutarate and increased 2-hydroxyglutarate strongly correlates with cancer formation. But the tumorigenic mechanism is not yet understood. One possibility may be that 2-hydroxyglutarate inhibits PHD activity by competing with α-ketoglutarate binding.
Solid or dashed lines indicate direct or indirect metabolic links, respectively.

oxidase activity. Furthermore, modeling α-ketoglutarate into the structure suggests a new orientation of the binding to α-ketoglutarate that can explain the formation of a new product, rather than simply running the reaction in reverse. Finally, Dang et al. demonstrated that 2-hydroxyglutarate levels are 100-fold higher in human gliomas that carry R132 mutations of IDH1 than in tumors with WT IDH1.

These results revealed a new gain-of-function activity of the tumor-derived IDH1 mutants and strongly correlated the levels of 2-hydroxyglutarate with tumorigenesis. However, does this grant 2-hydroxyglutarate the title "onco-metabolite" as Dang et al. proposed? What might be these oncogenic functions of 2-hydroxyglutarate?

The loss of activity of two other TCA cycle enzymes mentioned earlier, SDH or FH, supports tumor formation by increasing the levels of their respective TCA cycle substrates, succinate or fumarate. These substrates inhibit the oxygen-sensing enzymes hypoxia-inducible factor prolyl hydroxylases (PHDs) by competing with their cosubstrate α-ketoglutarate (MacKenzie et al., 2007). PHD inhibition leads to the activation of the HIF transcription factor among other, less characterized, effects (King et al., 2006). It was previously demonstrated that PHDs are inhibited in cells carrying mutant IDH1 (Zhao et al., 2009). Therefore, it is possible that like succinate and fumarate, 2-hydroxyglutarate inhibits PHD activity by competing with α-ketoglutarate (Figure 1). The observation that cell-permeable α-ketoglutarate esters prevent HIF activation in cells expressing mutant IDH1 (Zhao et al., 2009) supports this model.

The normal metabolic role of 2-hydroxyglutarate is not completely understood but 2-hydroxyglutarate is not unnatural to cells. It can be generated by specific α-ketoglutarate reductase enzymes (Struys, 2006) and oxidized back to α-ketoglutarate by 2-hydroxyglutarate dehydrogenases (2HGD) (Figure 1). The picture is further complicated by the existence of two enantiomers of 2-hydroxyglutarate with specific 2HGD for each. Mutations in 2HGD cause pathological accumulation of 2-hydroxyglutarate with different clinical features based on the enantiomer involved. Pathological accumulation of the L-2-hydroxyglutarate enantiomer is characterized by progressive neuronal defects and was recently linked to increased risk of brain tumors including gliomas (Aghili et al., 2009). This is strong support for the potential oncogenic role of 2-hydroxyglutarate, but with one caveat: Dang et al. demonstrated that mutant IDH1 generates D-2-hydroxyglutarate and not the L enantiomer. Accumulation of D-2-hydroxyglutarate is observed in D-2HGD-deficient patients and is associated with encephalopathy, cardiomyopathy, and more—but, so far, not with tumors (Struys, 2006). It is possible that D-2-hydroxyglutarate, when reaching very high levels, is too toxic to have tumorigenic potential. This could have therapeutic significance because it may suggest that a small and transient pharmacological inhibition of 2HGD, by raising the levels of 2-hydroxyglutarate from protumorigenic to toxic, could specifically kill gliomas with IDH1 mutations.

REFERENCES

Aghili, M., Zahedi, F., and Rafiee, E. (2009). J. Neurooncol. *91*, 233–236.

Dang, L., White, D.W., Gross, S., Bennett, B.D., Bittinger, M.A., Driggers, E.M., Fantin, V.R., Jang, H.G., Jin, S., Keenan, M.C., et al. (2009). Nature *462*, 739–744.

King, A., Selak, M.A., and Gottlieb, E. (2006). Oncogene *25*, 4675–4682.

MacKenzie, E.D., Selak, M.A., Tennant, D.A., Payne, L.J., Crosby, S., Frederiksen, C.M., Watson, D.G., and Gottlieb, E. (2007). Mol. Cell. Biol. *27*, 3282–3289.

Parsons, D.W., Jones, S., Zhang, X., Lin, J.C., Leary, R.J., Angenendt, P., Mankoo, P., Carter, H., Siu, I.M., Gallia, G.L., et al. (2008). Science *321*, 1807–1812.

Struys, E.A. (2006). J. Inherit. Metab. Dis. *29*, 21–29.

Xu, X., Zhao, J., Xu, Z., Peng, B., Huang, Q., Arnold, E., and Ding, J. (2004). J. Biol. Chem. *279*, 33946–33957.

Yan, H., Bigner, D.D., Velculescu, V., and Parsons, D.W. (2009a). Cancer Res. *69*, 9157–9159.

Yan, H., Parsons, D.W., Jin, G., McLendon, R., Rasheed, B.A., Yuan, W., Kos, I., Batinic-Haberle, I., Jones, S., Riggins, G.J., et al. (2009b). N. Engl. J. Med. *360*, 765–773.

Zhao, S., Lin, Y., Xu, W., Jiang, W., Zha, Z., Wang, P., Yu, W., Li, Z., Gong, L., Peng, Y., et al. (2009). Science *324*, 261–265.

SUMO Boosts the DNA Damage Response Barrier against Cancer

Jiri Bartek[1,2,*] and Zdenek Hodny[2]
[1]Institute of Cancer Biology and Centre for Genotoxic Stress Research, Danish Cancer Society, Strandboulevarden 49, DK-2100 Copenhagen, Denmark
[2]Department of Genome Integrity, Institute of Molecular Genetics, ASCR v.v.i., Videnska 1083, CZ-142 20 Prague 4, Czech Republic
*Correspondence: jb@cancer.dk
DOI 10.1016/j.ccr.2009.12.030

Cells exposed to genotoxic insults such as ionizing radiation activate a signaling cascade to repair the damaged DNA. Two recent articles published in *Nature* show that such genome maintenance requires modifications of tumor suppressor proteins BRCA1 and 53BP1 by the *s*mall *u*biquitin-like *mo*difier SUMO.

Proper genome maintenance, ensured by the cellular DNA damage response (DDR) machinery, is a prerequisite for normal development and prevention of premature aging and diverse devastating diseases including cancer (Jackson and Bartek, 2009). Indeed, one reason for cancer incidence not being even higher appears to be the intrinsic ability of our cells to detect and deal with the DNA damage caused by exogenous genotoxic agents such as radiation or chemicals as well as endogenous sources such as oncogene-evoked replication stress and telomere erosion during the early stages of cancer development (Halazonetis et al., 2008; Jackson and Bartek, 2009). Even if some DNA lesions, such as subsets of DNA double-strand breaks (DSB) that occur commonly during tumorigenesis, remain unrepaired, sustained signaling and effector pathways within the DDR "anticancer barrier" machinery usually eliminate such hazardous, genetically unstable cells by inducing cell death or a permanent cell cycle arrest known as cellular senescence (Halazonetis et al., 2008).

From the mechanistic viewpoint, sensing, signaling, and repair of DSBs involve a plethora of proteins whose sequential accrual and function at the DNA damage sites is modulated by a myriad of post-translational modifications, including phosphorylation, acetylation, methylation, and ubiquitylation, which are highly dynamic and reversible. The phosphorylation/dephosphorylation events are performed by kinases such as the ATM, ATR, and DNA-PK, and several protein phosphatases (Jackson and Bartek, 2009). The emerging ubiquitylation cascade comprises the E3 ubiquitin ligases RNF8, RNF168, and BRCA1, as well as the E2 ubiquitin-conjugating enzyme UBC13 and the candidate assembly factor HERC2 (Bergink and Jentsch, 2009; Bekker-Jensen et al., 2010). Unlike the classical role of ubiquitylation in triggering protein degradation, however, this ubiquitin-mediated pathway orchestrates protein-protein interactions on damaged chromosomes and recruitment of the key DNA repair factors 53BP1 and BRCA1 to DSBs, thereby promoting genomic integrity (Figure 1).

Despite the rapid progress in understanding the molecular basis of DSB signaling and repair, more surprises are in store for us in this lively area of research, as illustrated by two recent reports in *Nature* (Galanty et al., 2009; Morris et al., 2009). These exciting studies provide evidence for a key role of yet another protein modification, sumoylation (covalent attachment of the small proteins known as SUMO1, SUMO2, and SUMO3), in coordinating the DNA damage response to DSBs (Figure 1). Processes critical for cell fate decisions including survival and some aspects of DNA repair have been linked to the sumoylation pathway, particularly in yeast (Bergink and Jentsch, 2009; Branzei and Foiani, 2008; Hay, 2005). However, the involvement of the sumoylation pathway in DSB response and its functional interplay with the ubiquitylation cascade that controls recruitment of 53BP1 and BRCA1 are novel and very relevant for genome maintenance and protection against cancer.

So what is revealed by the two new studies? First, in a complementary series of immunofluorescence and live-cell imaging experiments, they show that the SUMO1 and SUMO2/3 conjugates, as well as the E1 (SAE1), E2 (UBC9), and E3 (PIAS1 and PIAS4) sumoylation enzymes, all rapidly accumulate at the sites of DNA